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Serum MMP-8 and TIMP-1 as prognostic biomarkers in gastric cancer

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Abstract

Despite gastric cancer being rare nowadays in Western countries, it remains one of the leading causes of cancer death worldwide. The course of the disease varies, so the individual gastric cancer patient's prognosis is difficult to determine. The need for new biomarkers is crucial. The aim of this study was to evaluate the prognostic value of serum matrix metalloproteinase-8, serum tissue inhibitor of metalloproteinase-1, and tissue matrix metalloproteinase-8 in patients with gastric cancer. Preoperative serum samples from 233 patients with gastric cancer were retrospectively analyzed. Serum levels of matrix metalloproteinase-8 were analyzed with immunofluorometric assay, and tissue inhibitor of metalloproteinase-1 levels were determined by enzyme-linked immunosorbent assay. We also determined the tissue expression of matrix metalloproteinase-8 in 276 gastric cancer samples by immunohistochemistry. Survival data and death causes came from patient records, the Population Register Center of Finland, and Statistics Finland. Patients with a low (<31 ng/mL) or high (>131 ng/mL) serum matrix metalloproteinase-8 level had a considerably unfavorable prognosis ($p = 0.002$). Those patients with a high (≥ 170 ng/mL) serum tissue inhibitor of metalloproteinase-1 level also had a poor prognosis ($p < 0.001$), and the latter remained significant in multivariable analysis (hazard ratio = 1.85; 95% confidence interval: 1.26–2.72; $p = 0.002$). The molar ratio of serum matrix metalloproteinase-8 and tissue inhibitor of metalloproteinase-1 levels with low (<0.07) or high (>0.30) molar ratios predicted a worse prognosis ($p = 0.020$). Tissue matrix metalloproteinase-8 did not influence prognosis. These results suggest that serum matrix metalloproteinase-8, tissue inhibitor of metalloproteinase-1, and the ratio of matrix metalloproteinase-8/ tissue inhibitor of metalloproteinase-1 may prove useful biomarkers for prediction of prognosis in patients with gastric cancer.

Keywords

Gastric cancer, MMP-8, TIMP, prognosis

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Introduction

Gastric cancer is the world's fifth most common cancer type, with almost one million new cases annually; it is the third leading cause of cancer-related death with annually approximately 723,000 deaths. The 5-year survival rate, even after curative surgery, is only about 30%.[1–3] Estimation of gastric cancer prognosis relies on the tumor node metastasis (TNM) classification; additional prognostic information may come from biomarkers.

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The matrix metalloproteinases (MMPs), proteinases participating in extracellular matrix (ECM) degradation, are structurally related but genetically differing zinc-dependent endoproteases that hydrolyze components of the ECM.[4] They play an important role in many steps of cancer development, regulating the microenvironment of the tumor by regulating cancer cell growth, differentiation, apoptosis, and immune surveillance. Increased expression and activation of certain MMPs occurs in many human cancers, and overexpression is often associated with poor prognosis.[5]

Although MMPs have traditionally been associated with cancer progression because of their ability to degrade the ECM, some MMPs have recently been identified as having anti-tumor properties and tumor-resistant functions. MMP-8, also known as collagenase-2, is produced mainly by neutrophils and has been involved in a variety of inflammatory conditions. MMP-8's protective role in cancer may operate through its ability to regulate the inflammatory response.[6,7]

Tissue inhibitor of metalloproteinase-1 (TIMP-1) is one of the naturally presenting inhibitors of MMPs. Although it is vital in inhibiting MMPs, it works independently in tumor invasion and metastasis. Several studies have shown that high TIMP-1 levels are associated with aggressive tumors and worse prognosis in malignancies, including gastric cancer.[8,9] ECM degradation is an essential step of cancer invasion and metastasis.[10] Basement-membrane proteolysis depends on a balance between activities of MMPs and their inhibitors.[11]

Gastric cancer is a highly malignant disease with an unfortunate prognosis. Clinical practice would benefit from having prognostic biomarkers to evaluate tumor behavior and tumor response to treatment. Here, we studied serum levels of MMP-8 and TIMP-1 and also the immunoexpression of MMP-8 in gastric cancer tissues in relation to clinicopathological parameters.

Methods

Patients

The study cohort comprised 313 gastric cancer patients who underwent surgery for histologically verified gastric adenocarcinoma at the Department of Surgery, Helsinki University Hospital, between 2000 and 2009. The surgical method was total gastrectomy for 153 (48.9%) cases and partial gastrectomy for 160 (51.1%). Of the 313, 228 (72.8%) were operated on with curative intent, whereas 85 (27.2%) underwent palliative surgery. Their median age was 67.4 (interquartile range (IQR): 57.1–76.6) and 161 (51.4%) were women. According to the 7th edition of the UICC classification, stage distribution was 62 (19.9%) stage IA-IB, 72 (23.1%) stage IIA-IIB, 115 (36.8%) stage IIIA-IIIC, and 63 (20.2%) stage IV patients. Lymph-node metastases appeared in 198 (65.6%) and distant metastases in 63 (20.1%). Of these 313 gastric cancer patients, 15 (4.8%) received preoperative treatment, and 125 (39.9%) postoperative adjuvant treatment (74 chemotherapy, 2 radiotherapy, and 50 both). Survival data and death causes until September 2017 came from patient records, the Population Register Center of Finland, and Statistics Finland.

The Surgical Ethics Committee of Helsinki University Hospital (Dnro HUS 226/E6/ 06, extension TMK02 §66 17.4.2013) and the National Supervisory Authority of Welfare and Health (Valvira Dnro 10041/ 06.01.03.01/2012) gave their permission to use the tissue samples without individual consent in this retrospective study.

Serum samples

The 233 blood samples from gastric cancer patients were collected within 24 days prior to surgery (range 0–24 days). Most of the samples (95.7%) were taken within 3 days prior to the gastric cancer operation. The samples were centrifuged, and serum and plasma components stored as aliquots at −80°C until analysis. Serum levels of MMP-8 were determined by time-resolved immunofluorometric assay (IFMA) (Medix Biochemica, Espoo, Finland) according to manufacturer's instructions; the detection limit for MMP-8 was 0.08 ng/mL.[12] Serum levels of TIMP-1 were detectable with the commercially available enzyme-linked immunosorbent assay (ELISA) kit according to manufacturer's instructions (Biotrak ELISA System; Amersham Biosciences, Buckinghamshire, UK) and the detection limit chosen was 1.25 ng/mL.[13] For calculation of MMP-8/TIMP-1 molar ratios, concentration units (ng/mL) were converted to molarity units (mol/l) by use of the molecular weights of MMP-8 and TIMP-1, respectively.[4]

Tissue samples and immunohistochemistry

Tissue samples from 283 patients were available; these formalin-fixed and paraffin-embedded surgical-tissue samples came from the archives of the Department of Pathology, University of Helsinki. Once de-identified, they were analyzed anonymously. An experienced pathologist re-evaluated all histological slides and then defined and marked areas representing the highest grade of each individual tumor. In total, four 1.0 mm cores from each tumor block: two from the invasive front and two from the tumor center were sampled and embedded in a new paraffin block with a semi-automatic tissue microarrayer (Tissue Arrayer 1,

Beecher Instruments Inc., Silver Spring, MD, USA) as described.[14] For immunohistochemistry, sections of 4 μm were cut and processed.

Sections were fixed on slides and dried for 12–24 hours at 37°C then were deparaffinized in xylene and rehydrated through step-by-step decreasing concentrations of ethanol to distilled water. For antigen retrieval, sections were treated in a PreTreatment module (Lab Vision Corp., Fremont, CA, USA) in Tris-HCl (pH 8.5) and in Tris-ethylenediaminetetraacetic acid (EDTA) (pH 9) buffer for 20 minutes at 98°C. Sections were stained in an Autostainer 480 (Lab Vision Corp., Fremont, CA, USA). Tissues were incubated with a specific polyclonal rabbit anti-human antibody[15,16] diluted to 1:400 overnight at room temperature. Tissues from the colon and breast served for positive staining control.

Scoring of immunoreactivity

MMP-8 immunoreactivity was successfully scored in 276 tumors, scoring based on cytoplasmic MMP-8 intensity in cancer cells as 0–3. Strong positive immunoreactivity was scored as 3, moderate positivity 2, weak positivity 1, and negative immunoreactivity as 0. Tissue samples were scored independently by two researchers (A.L. and J.H.) without knowledge of patients' clinical status or outcome data. Samples with conflicting scores were re-evaluated until consensus. Of the four cores, the one with the highest score served for further analysis.

Statistical analysis

To determine the significance of difference in biomarker median serum concentrations among gastric cancer

Table 1. Significance of the difference in MMP-8 and TIMP-1 serum concentrations and MMP-8/TIMP-1 molar ratio in 233 gastric cancer patients.

Clinicopathological variable	MMP-8 (ng/mL)		TIMP-1 (ng/mL)		MMP-8/TIMP-1 (molar ratio)	
	Median (IQR)	p-value	Median (IQR)	p-value	Median (IQR)	p-value
Age[a]						
<67	57.8 (31.5–106)	0.561	145 (128–166)	<0.001	0.18 (0.09–0.30)	0.034
≥67	50.8 (27.6–104)		175 (148–203)		0.13 (0.07–0.23)	
Gender[a]						
Male	52.4 (30.8–107)	0.997	156 (134–185)	0.973	0.15 (0.08–0.29)	0.943
Female	57.4 (30.6–100)		159 (132–188)		0.16 (0.09–0.26)	
TNM stage[b]						
IA-IB	50.4 (32.0–104)	0.929	156 (134–177)	0.201	0.17 (0.08–0.29)	0.978
IIA-IIB	52.0 (30.8–94.4)		155 (133–190)		0.14 (0.09–0.24)	
IIIA-IIIC	53.2 (27.6–112)		152 (132–177)		0.17 (0.08–0.31)	
IV	57.8 (29.4–127)		174 (135–201)		0.16 (0.08–0.22)	
Tumor classification (pT)[b]						
pT1	49.2 (32.0–127)	0.773	156 (132–189)	0.588	0.14 (0.07–0.29)	0.946
pT2	52.0 (33.6–84.4)		155 (136–175)		0.14 (0.09–0.25)	
pT3	56.8 (26.4–106)		152 (133–180)		0.13 (0.08–0.29)	
pT4	57.6 (36.2–117)		163 (131–200)		0.17 (0.08–0.27)	
Lymph node metastasis (pN)[a]						
pN0	55.0 (32.0–103)	0.689	151 (129–182)	0.383	0.17 (0.09–0.29)	0.489
pN1-3	52.2 (27.6–107)		156 (134–186)		0.15 (0.08–0.27)	
Distant metastasis (pM)[a]						
pM0	52.4 (30.8–104)	0.573	154 (132–180)	0.035	0.15 (0.08–0.29)	0.963
pM1	57.8 (29.4–127)		174 (135–201)		0.16 (0.08–0.22)	
Laurén classification[a]						
Intestinal	67.0 (36.0–116)	0.044	166 (144–192)	0.021	0.18 (0.10–0.30)	0.132
Diffuse	49.2 (26.4–95.2)		149 (129–180)		0.15 (0.07–0.26)	
Tumor size, cma						
≤6.0	53.6 (31.7–104)	0.999	151 (132–180)	0.250	0.16 (0.09–0.29)	0.774
>6.0	57.0 (26.7–112)		160 (136–191)		0.16 (0.08–0.27)	
MMP-8 immunohistochemistry[a]						
Negative	50.0 (27.6–104)	0.186	150 (129–177)	0.008	0.16 (0.08–0.27)	0.501
Positive	65.0 (36.2–106)		169 (143–190)		0.16 (0.09–0.28)	

MMP-8: matrix metalloproteinase-8; TIMP-1: tissue inhibitor of matrix metalloproteinase-1; IQR: interquartile range.
[a]Mann-Whitney U-test.
[b]Kruskal-Wallis test.

subgroups, the Mann–Whitney U-test and Kruskal–Wallis test were applied. Survival curves were constructed according to the Kaplan-Meier method and compared with the Log Rank test. Cancer-specific survival was calculated from date of surgery to date of death from gastric cancer or until September 2017. For serum biomarkers MMP-8 and TIMP-1, and the MMP-8/TIMP-1 molar ratio we determined optimal cut-offs by the aid of receiver-operating characteristic (ROC) curves and found them to identify suitable groups for survival analyses. For multivariable survival analysis, the Cox proportional hazard model had the following covariates entered: age, gender, stage, TNM classification, Lauréns classification, tumor size, serum level of MMP-8 and TIMP-1, the MMP-8/TIMP-1 molar ratio, and MMP-8 immunohistochemical expression. A p-value of <0.05 was considered statistically significant. All statistical analyses were performed with IBM SPSS Statistics version 22.0–25.0 (IBM Corporation, Armonk, NY, USA).

Results

Of the 233 serum samples, median MMP-8 level prior to gastric cancer surgery was 54.8 ng/mL (IQR: 30.8–105 ng/mL) and 156 ng/mL (IQR: 132–187 ng/mL) for TIMP-1. Median molar ratio of MMP-8 and TIMP-1 was 0.153 (IQR: 0.082–0.280). Serum levels of MMP-8 were higher in patients with intestinal cancer ($p = 0.044$, Mann–Whitney U-test). TIMP-1 serum levels were higher among patients over 67 years ($p < 0.001$), ones with metastasized disease ($p = 0.035$), with intestinal cancer ($p = 0.021$), and in samples with positive MMP-8 immunohistochemistry ($p = 0.008$). In addition, the MMP-8/TIMP-1 molar ratio was higher among patients under 67 ($p = 0.034$, Table 1).

Serum MMP-8 and TIMP-1 univariable survival analyses

When we investigated optimal cut-offs by the aid of ROC curves, we found them to provide suitable groups for survival analyses. For MMP-8, we used cut-offs of 31 ng/mL and 131 ng/mL, finding that patients both with low and with high serum MMP-8 levels had considerably worse prognosis than did patients with an intermediate MMP-8 level ($p = 0.002$, Log Rank test, Figure 1(a)). Patients with high serum TIMP-1 (cut-off 170 ng/mL) had worse prognosis ($p < 0.001$, Log Rank test, Figure 1(b)). We also investigated the molar ratio of serum MMP-8 and TIMP-1 levels and found by ROC curve analysis two cut-offs (0.07 and 0.30). Patients with a low or high molar ratio had worse prognosis than did those with an intermediate ratio ($p = 0.020$, Log Rank test, Figure 1(c)).

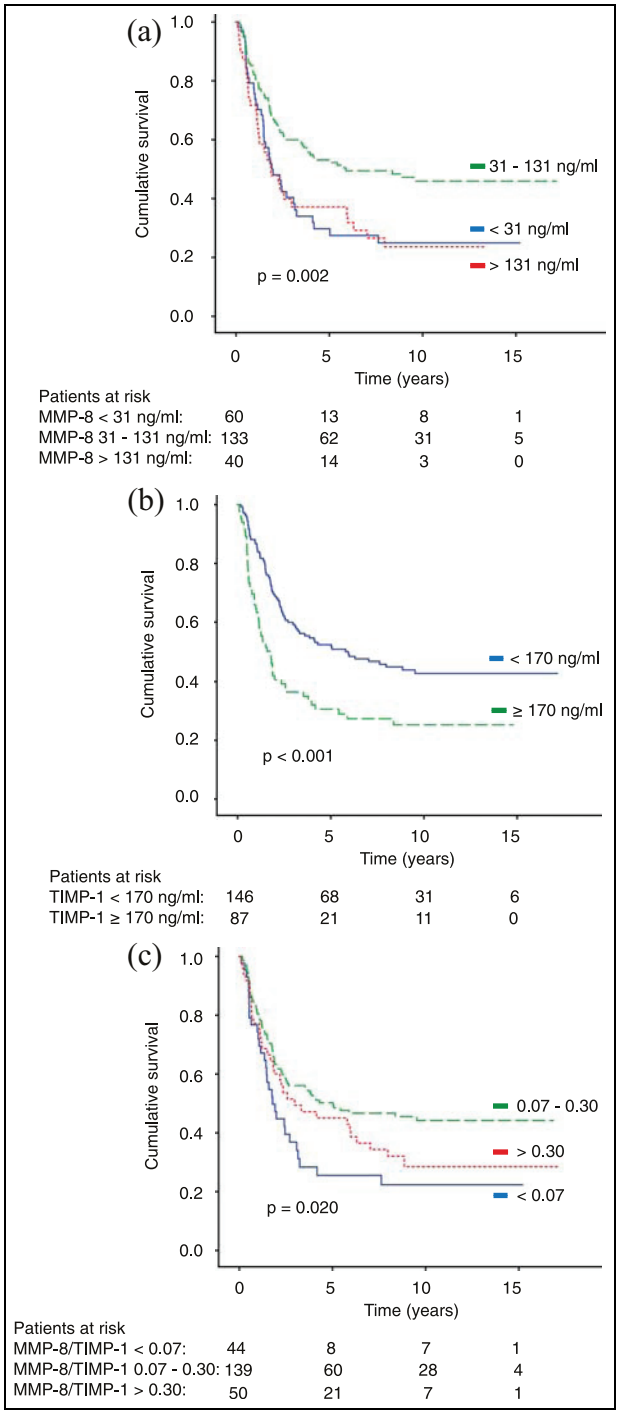

Figure 1. Cancer-specific survival in gastric cancer patients according to the Kaplan-Meir method. Serum levels of (a) MMP-8, (b) TIMP-1, and (c) MMP-8/TIMP-1 molar ratio. p-value for Log Rank test.

Serum MMP-8 and TIMP-1 subgroup analyses

In subgroup analyses, survival was favorable in patients with an intermediate MMP-8 serum level (31 to 131 ng/mL) in TNM stages I-II ($p = 0.003$, Figure 2(a)). In addition, stage III to IV patients with low TIMP-1

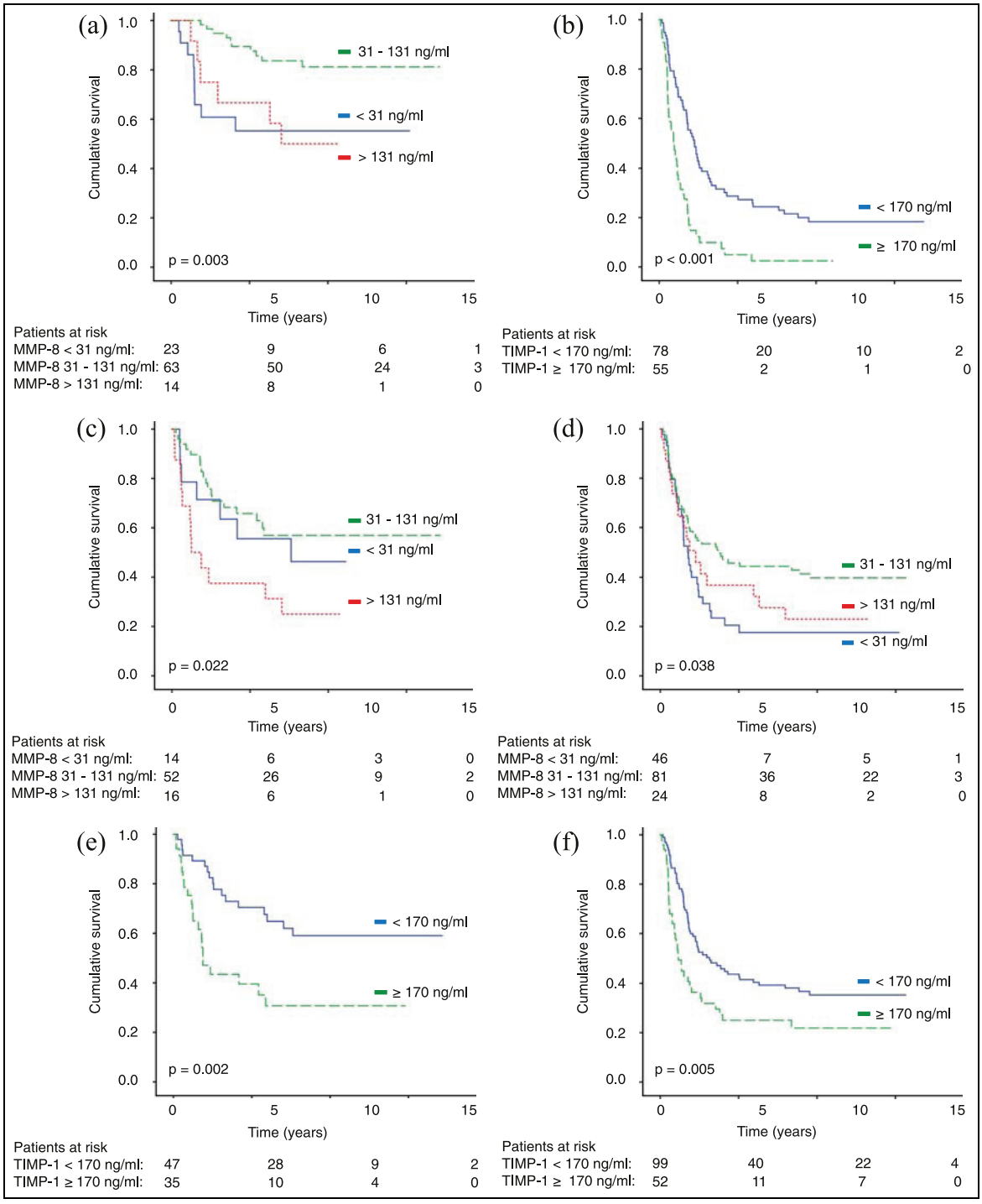

Figure 2. Cancer-specific survival of subgroups of gastric cancer patients according to the Kaplan-Meier method. Serum levels of (a) MMP-8 in patients with stage I-II disease, (b) TIMP-1 in patients with stage III-IV disease. Serum MMP-8 in patients with (c) intestinal, (d) diffuse type cancer. Serum TIMP-1 in patients with (e) intestinal, and (f) diffuse type cancer. p-value for Log Rank test.

levels (< 170 ng/mL) had a better prognosis ($p < 0.001$, Figure 2(b)). An intermediate MMP-8 level (31–131 ng/mL) and a low TIMP-1 level (< 170 ng/mL) were significant markers of better prognosis in both the intestinal type and the diffuse cancer type (Figures 2(c)–2(f)). Other subgroups studied together with the cumulative 5-year survival according to the Kaplan–Meier method are in Tables 2 and 3.

Table 2. Kaplan-Meier analysis for serum disease-specific survival stratified for serum MMP-8 concentrations in subgroups of gastric cancer patients. p-value for log-rank test.

Subgoups	5 year cumulative survival (95% CI)			p-value
	MMP-8 (ng/mL)			
	<31	31–131	>131	
Age, years				
<67	35.9 (18.1–53.7)	53.9 (42.3–65.5)	48.1 (26.7–69.5)	0.100
≥67	21.8 (4.36–39.2)	52.0 (38.3–65.7)	23.5 (3.31–43.7)	0.015
Gender				
Men	39.4 (19.4–59.4)	56.2 (43.5–68.9)	28.3 (7.33–49.3)	0.004
Women	22.2 (6.91–37.5)	50.2 (38.1–62.4)	45.0 (23.2–66.8)	0.087
TNM stage				
I-II	55.3 (33.4–77.3)	89.4 (81.4–97.4)	66.7 (40.0–93.4)	0.003
III-IV	14.0 (1.65–26.4)	20.1 (10.1–30.1)	23.1 (6.83–39.4)	0.719
Tumor classification (T)				
T1	87.5 (64.6–100)	94.4 (83.8–100)	83.3 (53.5–100)	0.655
T2	55.6 (6.99–100)	91.6 (80.4–100)	33.3 (0–86.6)	<0.001
T3	12.7 (0–26.2)	39.5 (23.4–55.6)	41.7 (13.9–69.5)	0.011
T4	17.0 (0–36.6)	21.2 (8.66–33.7)	23.5 (3.31–43.7)	0.696
Lymph node metastasis (N)				
N0	60.9 (36.4–85.4)	85.6 (75.0–96.2)	66.7 (35.9–97.5)	0.109
N +	17.0 (4.01–29.9)	38.6 (27.4–49.8)	30.8 (13.0–48.6)	0.037
Distant metastasis (M)				
M0	28.5 (14.8–42.2)	63.0 (53.6–72.4)	50.2 (31.8–68.6)	<0.001
M1	34.3 (31.4–65.5)	6.30 (0–17.9)	10.0 (0–28.6)	0.005
Laurén classification				
Intestinal	55.6 (28.9–82.3)	65.8 (51.7–79.9)	37.5 (13.8–61.2)	0.022
Diffuse	20.5 (7.56–33.4)	45.7 (34.7–56.7)	36.8 (16.6–57.0)	0.038
Tumor size				
⩽6 cm	48.5 (30.3–66.7)	73.0 (62.8–83.2)	43.5 (22.3–64.7)	0.001
>6 cm	6.10 (0–17.5)	23.1 (11.0–35.3)	29.4 (7.64–51.2)	0.735
MMP-8 immunohistochemistry				
Negative	35.3 (19.0–51.6)	56.4 (43.5–69.3)	33.7 (13.5–53.9)	0.040
Positive	8.30 (0–23.8)	47.0 (33.7–60.3)	30.8 (5.71–55.9)	0.010

MMP-8: matrix metalloproteinase-8, CI: confidence interval.

Serum MMP-8 and TIMP-1 multivariable survival analyses

In multivariable survival analysis, we found that age, TNM stage, Laurén classification, and high TIMP-1 level (≥170 ng/mL) served as independent prognostic factors (Table 4).

MMP-8 expression in tumor tissues

MMP-8 immunoreactivity was possible to score in 276 (97.5%) tissue microarray samples. Cytoplasmic immunostaining was negative in 157 (56.9%), weak in 85 (30.8%), moderate in 30 (10.9%), and strong in 4 (1.4%) cases. For the final analysis, we dichotomized the scores into negative (score 0) and positive (scores 1–3) immunostaining. For representative images of immunostainings, see Figure 3.

Association of MMP-8 tissue expression with clinicopathological variables

Negative MMP-8 immunostaining was associated with patient age under 67 ($p = 0.007$), with stage I cancer ($p = 0.022$), with tumor classification T1 ($p = 0.005$), with cancer without lymph node metastasis ($p = 0.016$), and with diffuse cancer type ($p < 0.001$, Table 5).

Tissue MMP-8 univariable survival analyses and subgroup analyses

No significant difference emerged in gastric-cancer-specific survival ($p = 0.178$). The 5-year survival rate for negative immunostaining was 46.3% (95% confidence interval (CI) 38.1–54.5) and for positive, 36.3% (95% CI 27.1–45.5, data not shown). In subgroup analyses, prognosis was better for those with negative

Table 3. Kaplan-Meier analysis for serum disease-specific survival stratified for serum TIMP-1 concentrations in subgroups of gastric cancer patients. P-value for log-rank test.

Subgoups	5-year cumulative survival (95% CI)		p-value
	TIMP-1 (ng/mL)		
	<170	≥ 170	
Age, years			
<67	54.0 (44.0–64.0)	28.9 (11.1–46.7)	0.012
≥ 67	48.6 (33.5–63.7)	31.6 (18.9–44.3)	0.038
Gender			
Men	54.3 (42.5–66.1)	32.5 (15.6–49.4)	0.024
Women	50.6 (38.8–62.4)	29.3 (16.0–42.6)	0.003
TNM stage			
I-II	79.6 (69.6–89.6)	76.9 (60.6–93.2)	0.705
III-IV	28.7 (18.3–39.1)	4.90 (0–11.4)	<0.001
Tumor classification (pT)			
pT1	95.0 (85.4–100)	81.8 (59.1–100)	0.190
pT2	83.6 (68.9–98.3)	63.5 (30.4–96.6)	0.124
pT3	32.1 (19.6–44.6)	29.6 (8.63–50.6)	0.665
pT4	34.3 (19.2–49.4)	6.70 (0–15.3)	<0.001
Lymph node metastasis (pN)			
pN0	76.3 (64.0–88.7)	79.7 (61.9–97.5)	0.809
pN +	41.5 (31.1–51.9)	14.0 (4.00–24.0)	<0.001
Distant metastasis (pM)			
pM0	57.5 (48.7–66.3)	41.1 (28.0–54.2)	0.018
pM1	16.8 (0–34.1)	9.10 (0–21.1)	0.016
Laurén classification			
Intestinal	70.5 (57.0–84.0)	39.5 (21.5–57.5)	0.002
Diffuse	43.7 (33.7–53.7)	25.0 (12.5–37.5)	0.005
Tumor size			
$\leqslant 6$ cm	68.2 (58.2–78.2)	49.3 (33.6–65.0)	0.024
>6 cm	28.0 (15.5–40.5)	10.9 (0.12–21.7)	0.002
MMP-8 immunohistochemistry			
Negative	50.0 (39.2–60.8)	35.4 (18.2–52.7)	0.164
Positive	51.6 (36.3–66.9)	24.1 (10.6–37.6)	<0.001

TIMP-1: tissue inhibitor of matrix metalloproteinase-1, CI: confidence interval.

Table 4. Multivariable Cox regression analysis of cancer-specific survival for gastric cancer patients.

	Hazard ratio	95% CI	p-value
Age, years			
<67	1.00		
≥ 67	1.95	1.32–2.87	0.001
TNM stage			
IA-IB	1.00		
IIA-IIB	7.06	2.41–20.7	<0.001
IIIA-IIIC	22.7	8.20–62.7	<0.001
IV	75.7	26.1–220	<0.001
Laurén classification			
Intestinal	1.00		
Diffuse	2.27	1.52–3.37	<0.001
TIMP-1 level, ng/mL			
<170	1.00		
≥ 170	1.85	1.26–2.72	0.002

TIMP-1: tissue inhibitor of metalloproteinases-1; CI: confidence interval.

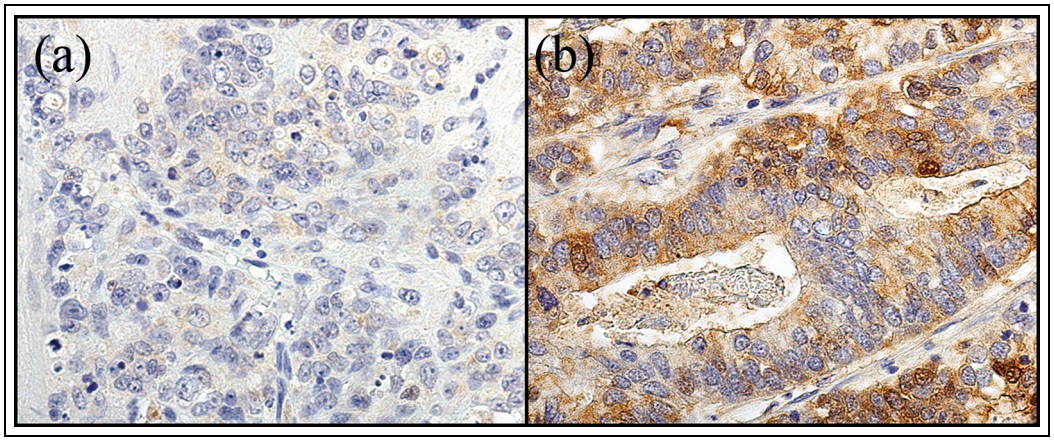

Figure 3. Representative images of MMP-8 immunostaining in gastric cancer tumors with (a) negative and (b) positive staining. Original magnification x40.

Table 5. Association of MMP-8 immunohistochemistry with clinicopathological variables in 276 gastric cancer patients.

Clinicopathological variable	n	MMP-8 immunostaining				p-value
		Negative		Positive		
		n	%	n	%	
Age						
<67	137	89	65.0	48	35.0	0.007
≥ 67	139	68	48.9	71	51.1	
Gender						
Male	134	78	58.2	56	41.8	0.666
Female	142	79	55.6	63	44.4	
TNM stage						
IA-IB	49	37	75.5	12	24.5	0.022
IIA-IIB	64	36	56.3	28	43.8	
IIIA-IIIC	104	51	49.0	53	51.0	
IV	58	32	55.2	26	44.8	
Tumor classification (pT)						
pT1	37	31	83.8	6	16.2	0.005
pT2	41	23	56.1	18	43.9	
pT3	87	45	51.7	42	48.3	
pT4	111	58	52.3	53	47.7	
Lymph node metastasis (pN)						
pN0	87	58	66.7	29	33.3	0.016
pN1-3	180	92	51.1	88	48.9	
Distant metastasis (pM)						
pM0	218	125	57.3	93	42.7	0.767
pM1	58	32	55.2	26	44.8	
Laurén classification						
Intestinal	111	48	43.2	63	56.8	<0.001
Diffuse	165	109	66.1	56	33.9	
Tumor size, cm						
$\leqslant 6.0$	149	91	61.1	58	38.9	0.069
>6.0	120	60	50.0	60	50.0	

MMP-8: matrix metalloproteinase-8.

MMP-8 immunostaining among women ($p = 0.026$) and among those with low (<31 ng/mL) serum MMP-8 ($p = 0.018$, data not shown). When MMP-8 immunostainings were analyzed as two subgroups, negative and positive, we found that intermediate (31–131 ng/mL) serum MMP-8 level was a significant marker of better prognosis in both subgroups ($p = 0.040$, $p = 0.010$; Supplementary figure A-B). In addition, low

(<170 ng/mL) TIMP-1 level was a significant marker of better prognosis only in the subgroup of MMP-8-positive immunostaining ($p < 0.001$), not in MMP-8-negative ($p = 0.164$, Supplementary figure C-D).

Discussion

Here, we show, to our knowledge for the first time, that gastric cancer patients with either low or high preoperative serum MMP-8 value had a considerably worse prognosis, and we also strengthen the findings that patients with elevated serum TIMP-1 levels have a poor outcome.

Interestingly, a worse prognosis was evident both in patients with low MMP-8 level and in those with a high level compared with those with an intermediate MMP-8 level. This phenomenon has not yet been described in a clinical patient series. MMP-8 takes part in many biological processes and is shown to also have antitumor properties. MMP-8 deficient mice have shown to develop skin tumors and tongue cancer more often than wild type mice.[6,7] In addition, in breast cancer cells, MMP-8 expression causes a decrease in tumor growth and lung metastasis formation proving an evidence of MMP-8 antitumor function in cancer and metastasis.[17] Korpi et al. showed also that in a clinical patient cohort of tongue cancer, MMP-8 expression is significantly linked with prolonged survival.[7] In contrast, high MMP-8 levels have also been associated with advanced cancer type and poor patient outcome in hepatocellular and colorectal cancer.[18,19]

Our serum MMP-8 findings differ from those of most markers that have only one cut-off dividing the patients into those with poor or with good prognosis. Our results indicate that a normal physiological level of MMP-8 is the most favorable for the patient and that either lack of MMP-8 or excess MMP-8 favors cancer aggressiveness. The role of MMP-8 seems to be more complex than that of other MMPs in various tissues.

In multivariable analysis, high serum TIMP-1 level served as an independent marker of worse prognosis. TIMP-1 plays an independent role in maintaining the balance between ECM deposition and degradation in healthy and malignant tissues. In cancer, TIMP-1 can exert an effect on tumor growth, invasion, and metastasis.[20] Thus, our finding strengthens the earlier findings in gastric cancer.[8,9] Earlier, only one gastric cancer study before ours showed that patients with elevated TIMP-1 levels had poor prognosis when using disease-specific survival as an endpoint as we did.[21] Thus, our study strengthens knowledge concerning TIMP-1 as a biomarker of poor prognosis in gastric cancer.

As we measured, the serum levels of both MMP-8 and TIMP-1, knowing how these two interact in tissues when TIMP-1 binds MMPs in a 1:1 stoichiometry, it was interesting to calculate also the molar ratio of these two markers. After the nonlinear MMP-8 result, it was reasonable also to find two cut-offs for their molar ratio, and patients with low or high molar ratio had worse prognosis than those with intermediate ratio.

Interestingly, tissue MMP-8 did not influence prognosis. This finding indicates that the same biomarker's serum level and tissue expression do not necessarily correlate. Differences occur in how it is expressed in tumor tissue and the amounts of the active protein in circulation. In gastric cancer, the active serum MMP-8, we measured may originate in different sources related to cancer rather than originating in the tumor tissue itself. In addition, serum or plasma levels of MMP-8 are also elevated in certain cardiovascular diseases, in *Helicobacter pylori* infection, and in periodontitis.[12,22–26]

The strength of this study is a large gastric cancer patient population with both serum and tissue microarray samples, and with reliable and lengthy clinical follow-up data. The tissue microarray method allows analysis of only small spots from each tumor, but on the other hand, the whole tumor cohort can be stained as one batch without risk of variation between staining series; moreover, this method makes it possible to evaluate a large number of patient samples in a moderate length of time.

In conclusion, both serum MMP-8 and its inhibitor TIMP-1 are promising prognostic markers for gastric cancer. MMP-8 tissue expression had no prognostic value. Our results are promising but need to be validated in other patient cohorts.

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